IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 71007/137/USGO

In re patent application of
Apurba BHATTACHARJEE et al.
Serial No. 08/230,402
Filed: April 20, 1994
For: VACCINE AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS

Group Art Unit: 1641
Examiner: S. Loring

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Alan S. Cross, M.D., declare and say as follows:

1. I am the Alan S. Cross shown as coinventor on the captioned patent application.

2. I am experienced in the field of vaccines directed against bacterial infections. My curriculum vita is appended to my prior declaration.

3. Zollinger discloses vaccines comprising outer membrane protein and polysaccharide, in which the polysaccharide portion of the vaccine can be capsular polysaccharide or lipopolysaccharide (LPS) of any Gram-negative bacteria, with *E. coli* being just one of the possibilities. By contrast, the present claims recite combinations of outer membrane protein (OMP) derived from *N. meningitidis* and purified, detoxified LPS endotoxin derived from a particular mutant strain of *E. coli* that lacks O-polysaccharide sidechains, the J5 strain.

4. Combinations of OMP derived from *N. meningitidis* and purified, detoxified LPS endotoxin derived from *E. coli* strain J5 provid unexpectedly superior protection against gram-negative sepsis as compar d to combinations of OMP with LPS purified, detoxified endotoxins from other strains of *E. coli*.

In our studies, we have complexed OMP derived from *N. meningitidis* with other lipopolysaccharides, including LPS endotoxin derived from a strain of *Brucella* and from *E. coli* 018 (EC018). Ten mice/group were immunized with PBS or with 20 µg of vaccine (OMP, Brucella-OMP, J5-OMP, or EC018-OMP) at day 0, day 14 and day 28. EIA against all vaccine antigens, lipid A and Re LPS was done on sera drawn prior to challenge. Seven days after the immunization protocol was completed, the mice were challenged with 100 ng of EC018 lipopolysaccharide and 20 mg galactosamine intraperitoneally. Thus, challenge was homologous with respect to mice immunized with EC018-OMP, while challenge was heterologous with respect to mice immunized with J5-OMP.

5. There were no survivors among mice immunized only with OMP. No protection was provided by immunization with *Brucella*-OMP. Some protection was expected for mice immunized with EC018-OMP, since challenge was with the homologous strain. Sixty percent survival (p=0.01) was observed in the group of mice immunized with EC018-OMP. In mice immunized with J5-OMP, however, survival was 90% (p=0.0001), i.e., vaccination with J5-OMP provided 50% greater protection than vaccination with EC018-OMP. This was particularly surprising in view of the fact that J5-OMP vaccine was providing protection against infection by a heterologous strain (EC018) whereas EC018-OMP was providing protection against the same strain. LPS endotoxin from *E. coli* J5 in combination with OMP from *N. meningitidis* clearly provides protection that is markedly superior to LPS endotoxin from other strains of *E. coli* in combination with OMP from *N. meningitidis*.

6. A clinical protocol to test the safety of the present vaccine for immunizing a subject against infection by heterologous Gram-negative bacteria or against lipopolysaccharide (LPS) endotoxin-mediated pathology has been written for Phase I trials in humans, and has been approved by (1) the Walter Reed Army Institute of Research (WRAIR)

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Scientific Review Committee; (2) the WRAIR Institutional Review Board (IRB); and (3) the Surgeon General's Human Subjects Research Review Board (pending only the formality of my being credentialed at WRAIR so that I may act as principal investigator). My co-inventor Dr. Bhattacharjee has consulted with Dr. Richman of the FDA about the specifics of the protocol, who suggested minor modifications to the Phase I trial. These modifications were incorporated, and the protocol will be submitted, along with the IND application, to the FDA.

I further declare that all statements made in this declaration of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of any patent that may issue based on them.

Respectfully submitted,

June 19, 1998
Date

Alan S. Cross, M.D.